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CHEMICAL CHARACTERIZATION OF GOMUTRA (GIR) BY HIGH RESOLUTION HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

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ABSTRACT:

Cow urine has lot of application in medicine Cow urine provides immunity power by increasing resistance power against diseases in human body. It is anti toxin. It has been found that cow urine has potential to control amongst cow urine is best their entire exercise of marketing the cow products and promising huge economic benefits. Collected the (Gir) cow urine Gir Fresh, Distillate and Go-Kshar these all are under study of HPLC (High Performance Liquid Chromatography) HPLC performed on LC-8A shimadzu HPLC with mobile phase water acetonitrile (80:20) flow rate 1.0/min uv detection at 275 nm and C-18 E MERCK (150X4nm) retention time 20 min at the end of the result the chemical compound are identified.

- 1) 3-acetylgedunol
- 2) 5 alpha-pregnan-3-one, 11,20-dione.
- 3) 3,8 diazabicyclo (3.2.1) octane, 3-(6-chloro-3-pyridazinyl
- 4) 2-(4-(3-acetyl-3-hydroxy-2-propylphenoxy)propoxy)propoxy)acetic acid.

KEY WORDS: Gir-Cow urine, Distillate, Go-Kshar, HPLC.

INTRODUCTION:

Cow were regarded as wealth and where the backbone of the economy of ancient Indians. Wars were fought for acquiring cows, cattle were one of the most frequency used animals described in Vedas cow were regarded as mother “Gau-mata” and referred to as agnya. Prayers were offered to agni (God of fire) to kill with his flame all those evil dweller, who stole milk of cows, voluminous prescribe cow urine for worm. Complaints to develop immunity and to avoid ageing (*Edwin jarald, sheeja*

Edwin *et.al.*, 2008). In veda cow's urine was compared to the nector (*Rig veda*). cow's urine is known to cause weight loss and reverse certain cardiac and kidney problems, indigestion, stomach ache, edema, ring worm, itching, psoriasis, gynae and such disease etc., (K.Krushnamurthi *et al.*, 2004), cow urine has amazing germicidal power to kill varieties of germs. All germs generated disease are thus destroyed cow urine corrects functioning of liver cow urine contain many minerals especially copper, salt, etc.

Cow urine is called (*medhya and hradya*) which means it gives strength to brain and heart "cowpathy" has been reported to be beneficial even for dreaded disease like cancer, aids and diabetes practitioners of Ayurvedic medicine from india routinely use cow urine has a remedy and the medicine made from it are used to cure several disease. The various products of cow urine were used in medicine from ancient period cow urine has disinfectant antiseptic and it was also found antimicrobial, antiniotics, and microbial activity (Shiv kumar, D.singh, *et.al.*, 2013)

Another part of the problem is due to increasing use and misuse of exting antibiotics in human and veterinary medicine and also in Agriculture. Now a days about 70 % the bacteria that cause infection are resistant to at least of the drug which most commonly used for the treatment cow urine control the bacterial disease the purpose of this study was to deremine antibacterial activity (K.Rajapandiyam, S.Shanthi *et al.*, 2011). Experimentally it has also been proved that among urine from various species the Indian cow is most effective of its medical properties (Subha ganguly 2013). The gir animals are famous for their tolerance to sters conditions and resistance to various tropical diseases. Cow urine has got application in agriculture it has been found that cow urine has potential to control. Cow urine has been used in preparations of hair oil, shampoo, skin cream, soap, nasal power, body power, body cream, tooth power, etc., (cow urine :principles and applications). Amongst urines cow urine is best this entire exercise of marketing the cow's products and promising huge economic benefits. The analysis of cow urine has shown that it contain nitrogen, sulphur, phosphate, sodium, manganese, carbonic acid, iron, silicon, chlorine, chloline, magnesium, citric acid, succenic, calcium, salt, vitamin a, b, c, d, e, minerals, lactose, enzymes, hormones, and gold acid.(N.K jain *et al.*, 2012) According to the Chinese pharmaceutical dictionary "shang han lun " urine has been used to get her economized the quantity of precious medicinal herbs.(Nidhi Garg and R.S.Chauhan *et.al.*, (2005). Cow pathy is an old system of medicine mentioned in acient Indian literature (Ayurveda) as panchgavya chikitsa.

MATERIALS AND METHOD:

PREPARATION OF FRESH COW URINE:

Cow urine was collected from healthy cows of Gir breeds (six cows) free from any infection. Maintained under medical supervision at goshala in sri-vital-rukmini samasthan Govindhapuram. Three litre (pored sample of six cows) of cow urine collected in steril container and was transpored immediately to the laboratory.

PREPARATION OF COW URINE DISTILLATE:

Cow urine was distilled at 40 c using distillation apparatus. The urine was again distillate at 40 c using distillation apparatus to remove ammonia. The distillate was stored in sterile glass flask at refrigerator at 4 c.

PREPARATION OF COW URINE GO-KSHAR:

The remaining cow urine after distillation is called go-kshar or salt of cow urine. It will be collected separately and stored in a sterile glass container.

METHOD OF HPLC:

HPLC was performed on LC-8A shimadzu HPLC with mobile phase water acetonitrile (80:20), flow rate 1.0ml/min, uv dection at 275 nm and C-18 E MERCK (150X4mm) coloum, rendition time 20 min.

RESULT

FRESH COW URINE:

The fresh peak % is 91.312 and the rendition time is 3.218 identified compounds is 3-acetylgedunol. Height of the peak is 90.580 two peak are identified from the fresh urine sample second peak % is 8.688 and rendition time is 5.855 identified compound is 5 alpha-pregnan-3 alpha-01-11,20-dione. The total peak % is 100.000

COW URINE DISTILLATE:

During the analysis the distillate peak % is 21.261 and the retention time is 3.805 identified compound is 3,8 diazabicyclo (3.2.1) octane, 3-(6chloro-3-pyridazinyl). Height of the peak is 31.992 two peak are identified from the distillate sample second peak % is 78.739 and retention time is 4.303 identified compound is 2-(4-(3-4 acetyl-3-hydroxy-2-propylphenoxy) propoxy)propoxy)acetic acid. The total peak % is 100.000.

GO-KSHAR:

Go-kshar peak % is 80.509 and retention time is 3.227 identified compound is 3-acetylgedunol. Height of the peak is 78.125 two peak are identified from the sample go-kshar. The peak % is 19.491 and retention time is 4.900 identified compound is 2-(4-(3-4 acetyl-3-hydroxy-2-propylphenoxy) propoxy) propoxy) acetic acid. The total peak % is 100.000.

The first compound for fresh and go-kshar is same 3-acetylgedunol they have no known the activity. And distillate and go-kshar second peak is same the compound are 2-(4-(3-4 acetyl-3-hydroxy-2-propylphenoxy)propoxy)propoxy)acetic acid they have biological activity they are discussed the table.

DISCUSSION:

Cow urine has popular for its anti microbial properties. In traditional medicine and siddha the usage of more cow urine is used that they are not producing any side effect, when it is introduced in a scientific manner the cow urine sample is analyzed six different compounds present with HPLC analysis by fixing the time 0 min to 36 min.

In the fresh cow urine the peak % are 91.312, 8.688, and the compound present in the cow urine was 3-acetylgedunol, they are no activity are present, and in the second peak 5 alpha-pre-ghan-3-alpha-01,-11,20 done are present they have the 5a pregnane 3,20 dione is a biologically activity 5 alpha reduced metabolic of plasma progesteron. It is the immediate precursor of 5 alpha pregnan, 3alphaol 120 one (allopregnanolone), a neuro active steroid that bind with GABA receptor are generally composed of cell or organs which are destined to receive only one kind of stimulus and not any other.

GABAA receptor is an ionotropic receptor and ligand, gated ion channel. GABA receptor is widely distributed and utilized throughout the CNS early GABA ergic drugs had very generalized effect on CNS function. The development of more selective agents had led to the identification of at

least two distinct classes of GABA receptor GABAA and GABAB. The GABAA receptor is the major molecular target for the action of many drugs in the brain. Biological function of cell signalling is part of a complex system of communication that governs basic cellular activities and coordinates cell actions. Biological function cell signalling fuel and energy storage fuel or energy storage. Fuel or energy become hormones membrane component membrane integrity/stability.

In distillate the HPLC analysis peak % was 21.261, 78.739 the compound present in this distillate (CUD) WAS 3,8 diazabicyclo (3.2.1), octane, 3-6 chloro-3-pyridazinyl) they have the activity based on the structure characteristics of the lead compound 1, octanedioyl 14,4' dimethyl 14,4' dibenzyl, dipiperazinium dibromide (2) and 3,8 disubstituted 3,8 diazabicyclo (3.2.1) octanes (DBO), distillate (3,8 diazabicyclo (3.2.1) octane, were evaluated for their in vivo analgesic and sedative activities piperazine is an organic compound that consists of a six membered ring containing two nitrogen atoms at opposite in the ring piperazine exists as small alkaline deliquescent crystals with saline taste it has core piperazine function group lead compound 2 causes loss of the analgesic activity and increase the toxicity dramatically piperazine in compound interaction with the receptors and quaternization of compound 10mac is the main reason for toxicity increase.

Diquaternary ammonium salts 3 ac were designed and synthesized through highly practical procedure sedative activities. Interestingly the result shows flexible conformation of piperazine in compound 2 interaction with the receptor main reason for the toxicity increase. In the distillate the second peak shows the 2-(4-(3-(4 acetyl-3-hydroxy 2-propylphenoxy) phenoxy)phenoxy). Acetic acid the activity of the compound raises plasma cholesterol levels in insulin resistant db/db mice and human being cerebral infarction and parkinson's disease are known as idiopathic or primary parkinsonism hypokinetic rigid syndrome it is degenerative disorder of the central nervous system disease results from the death of dopamine generating cells in the substantia nigra a region of the mid brain the cause of cell death is unknown.

Gok shar peak % was 80.509 and 19.491, 3-acetyldedunol they have no activity the second peak 2-(4-(3-(4 acetyl-3-hydroxy-2-propylphenoxy)propoxy)propoxy) acetic acid they have the activity of raises plasma cholesterol levels in insulin resistant db/db mice and is neuroprotective in models of cerebral infarction and parkinson's disease, above this study are analysed by HPLC on LC-8A shimadzu with mobile phase water acetonitrile (80:20) flow rate 0.1 ml/min uv detection at 275nm and c-18 EMERCK (150x4mm) column, retention time (20 min) . Experimentally it has also been

proved among urine from various species the cow is most effective of its medical properties (subha ganguly 2003). These compounds are found to be pharmalogically active like antimicrobial, anesthetic cancer preventive etc. This main objectives of this proposed proposal find out the cow urine is the best even that distillate is more effective than fresh and go-kshar.

Recently a pharmaceutical composition comprising an effective amount of bioactive fraction from cow urine distillate (CUD) as a pharmaceutically acceptable additive was tested and applied for a US patent. The invention relates to an absolutely novel use of CUD as an activation enhancer and availability facilitator for bioactive molecules such as anti-infective and anticancer drugs. It was found that the urine distillate effectively reduced the dosage of drug needed for the therapies. The applicants (Khanuja *et al.*, 2002) obtained the 'Kamadhenu Arka' (CUD) from Go-Vigyan Anusandhan Kendra, Nagpur, India and studied its effect and of the dried fraction (GM-IV). A pharmaceutical composition comprising of at least one anticancer agent ('Taxol'- Peclitaxel) and a cow urine distillate or a dried fraction (GM-IV) obtained from cow urine distillate was studied.

Cow urine has been recorded for its various bioenhancing properties in mice. The redistillate of cow's urine was found to possess total antioxidant status of around 2.6 μmol , contributed mainly by volatile fatty acids (1500 mg/L) as revealed by the GC-MS studies. These fatty acids and other antioxidants might cause the highly protective effects (Krishnamurthi *et al.*, 2004).

Scientists have proved that the pesticides even at very low doses cause apoptosis (cell suicide) in lymphocytes of blood and tissues through fragmentation of DNA. Distilled cow urine protects DNA and repairs it rapidly as observed after damage due to pesticides (Ambwani, 2004). It protects chromosomal aberrations by mitocycin in human leukocyte (Datta, 2001). Cow urine helps the lymphocytes to survive and not to commit suicide (apoptosis). Kumar *et al.* (2004) reported the prevention of pathogenic effect of free radicals through cow urine therapy. These radicals cause damage to various tissues and attack enzymes, fat and proteins disrupting normal cell activities or cell membranes, producing a chain reaction of destruction leading to the ageing process of a person. By regular use of cow urine one can get the charm of a youth as it prevents the free radicals formation.

Cow urine is an aqueous solution of nitrogenous and sulphurous compounds, minerals, and other minor components (Church, 1976). Concentrations of carbon (C) in cow urine are low (Lovell and Jarvis, 1996), and measurements of 3.1–20.4 g C L⁻¹ (mean = 9.9) have been made (Roger Cresswell, pers. comm., 2009). The pH of ruminant urine is usually between 8.4 and 8.6 (*e.g.* Sherlock and Goh, 1984; Haynes and Williams, 1992; Somda *et al.*, 1997), but may be as low as 7.2 (Doak,

1952). In ruminants, phosphorus (P) is predominantly excreted in the dung, and urine generally contains much less than 0.1 g P L⁻¹ (Betteridge et al., 1986; Wu et al., 2000; Knowlton and Herbein, 2002). Trace elements from the diet are not absorbed by the digestive tract so are expelled primarily in the dung; these include copper (Cu), zinc (Zn), manganese (Mn), iron (Fe), (Safley et al., 1984; Underwood and Suttle, 1999), cadmium (Cd) (Smith, 1984) and lead (Pb) (Reichlmayr-Lais and Kirchgessner, 1984). Excretion of molybdenum (Mo) via the urine increases when the diet is high in S (Barrow).

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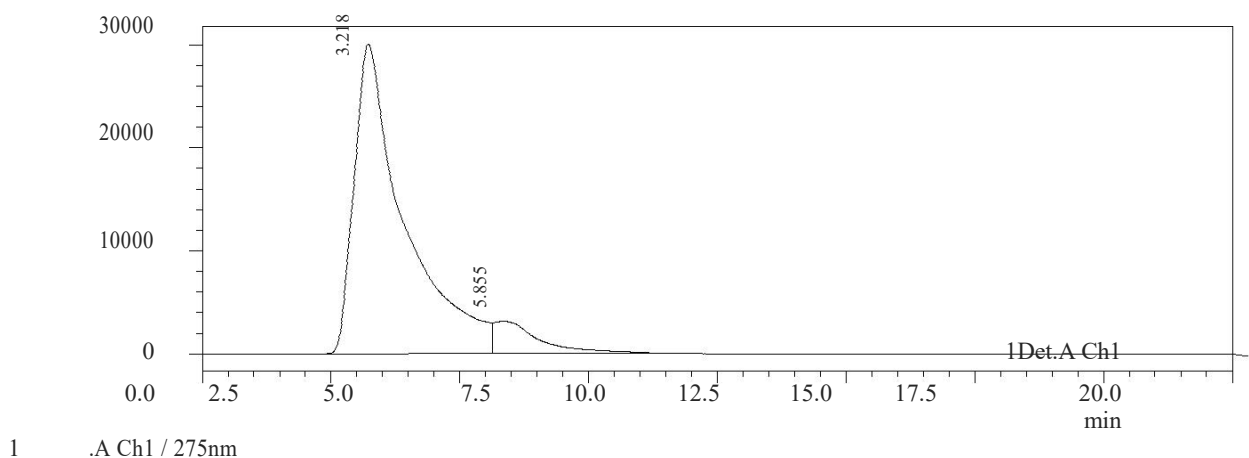
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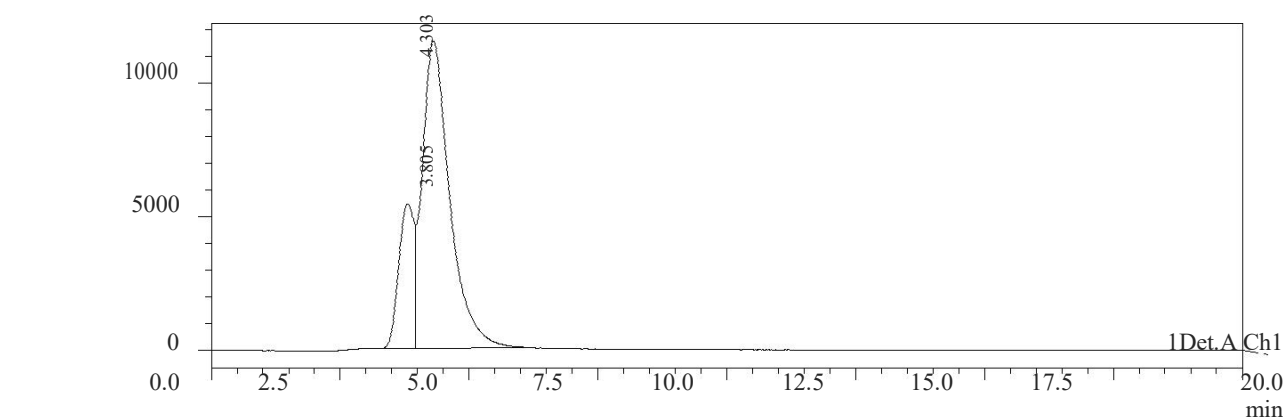
Figure 1: HPLC analysis of Fresh Gir Cow urine



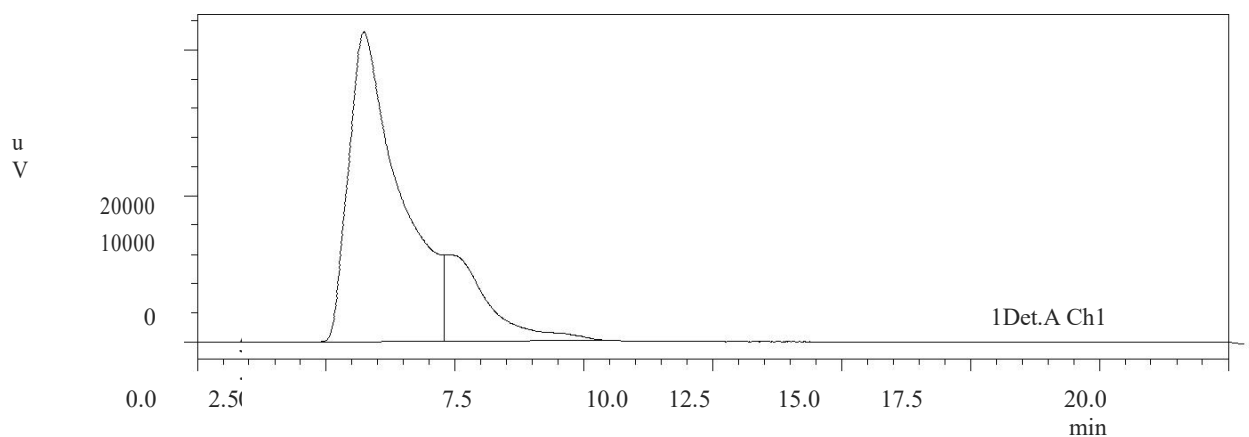
1

.A Ch1 / 275nm

Figure 2: HPLC analysis of Distillate Gir Cow urine



1 Det.A Ch1 / 275nm



1 Det.A Ch1 / 275nm

Table for fresh cow urine:

Peak#	Ret. Time	Area	Height	Area %	Height %
1	3.218	2039281	30073	91.312	90.580
2	5.855	194042	3128	8.688	9.420
Total		2233323	33200	100.000	100.000

Table for distillare :

Peak#	Ret. Time	Area	Height	Area %	Height %
1	3.805	114518	5412	21.261	31.992
2	4.303	424108	11505	78.739	68.008
Total		538626	16918	100.000	100.000

Table for go-kshar:

Peak#	Ret. Time	Area	Height	Area %	Height %
1	3.227	1463929	21209	80.509	78.125
2	4.900	354420	5939	19.491	21.875
Total		1818348	27147	100.000	100.000